ONYCHOMYCOSIS

An Experimental Study*

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Onychomycosis is undoubtedly among the most obscure subjects of dermatology. So far, the following problems have not been solved: 1) The fact that it is a relatively uncommon disease. 2) Its prolonged clinical course and resistance to treatment. 3) The mechanism by which infection occurs and its clinical features in the initial stages. 4) The histological structure of the lesions and the topographic distribution of the fungi infesting the nails. 5) The reason for the fact that even the most careful examination for fungi is frequently negative. 6) The difficulties involved in determining whether or not the fungi isolated from the infected nails have played a part in the pathogenesis.

1. The statistical studies by Heller (1) and Miescher (2) show that, with a few exceptions, all authors regard onychomycosis as a fairly uncommon disease, although naturally there are some marked regional variations.

The fact that infection of the nails is a rare event is also obvious from the few lesions of the nails appearing in subjects affected with fungus infections running a course so prolonged as that of favus, exposing the patient to infection over a prolonged period.

Our records show that during a period of eight years we observed 10 cases of onychomycosis (verified by microscopic and cultural examination) in 24,562 cases of skin disease, 2,363 of which were examined for possible fungus infection.

2. Nor has any satisfactory explanation been suggested to account for the fact that spontaneous cures hardly ever occur, that these diseases run an extremely prolonged course and that they are resistant to even the most vigorous methods of treatment.

3. Opinions as to the mechanism by which the nails are infected differ widely. Brocq (3), Gougerot (4), Fuhs and Kuhmer (5) and others believe that the infection originates from the free margin, the parasite penetrating between the nail bed and the body of the nail, giving rise to hyperkeratosis beneath the latter and finally raising the nail. There is only secondary invasion of the body of the nail. All this explains why the most marked changes occur at the distal extremity of the nail and its bed, whereas lesions fail to appear in the lunula over a prolonged period, provided that the rate at which centripetal invasion by the parasite occurs does not exceed the rate at which the nail grows (Hodges (6)).

Darier (7), Miescher (2) and Negroni (8) believe that the infection, in addition to arising from the free margin, may also originate from the lateral margins of the nail.

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Received for publication March 16, 1956.

Pellizari (9) assumes that the body of the nail may be directly invaded by the parasite. According to Ormsby and Montgomery (10) this will result in Jessner's (11) so-called leukonychia trichophytica. This mechanism is undoubtedly operative in Rost's (12) leukonychyia mycotica.

Stühmer (13) has described a form of epidermatophytosis, trichophytosis and favus, called subungual as the lesions are localized beneath the nail in which infection occurs after the parasite has passed round the eponychium and the root of the nail. These cases are marked by the appearance of white spots, which may occur successively; they are arched, sharply defined, parallel to the free margin of the nail and in front of these spots a very narrow congested rim may be observed. Examination under the magnifying glass and lateral illumination shows the spots to be localized exactly under the body of the nail. Originating in the lunula, these spots spread towards the free margin and they may be the starting-point for a process of decay of the distal portion of the nail, due to softening and the presence of air.

Possibly, this form of infection is also related to the lesions described by Alkiewicz (14), which consist in a delicate white network (leukopathia unguis mycotica) arranged parallel to the transverse axis of the nail and detectable under the magnifying glass after the body of the nail has been made transparent with a drop of cedar oil.

4. Few reports concerned with histopathological studies on the lesions occurring in fungus infections of the nails are available, despite the statement by Sulzberger and Baer (15): "how much can be learned and is still to be discovered through careful histopathologic studies of diseases of the nails." Undoubtedly the difficulties involved in a complete histological study of the affected nails should be taken into account, as this would require total amputation of the terminal phalanx (Pardo Castello (16)). Owing to the extreme fragility of the subungual tissues, current biopsy inevitably causes excessive injury, a fact also pointed out by Stühmer (13).

Unna (17), who was the first to engage in histological studies on onychomycosis, states that in favus the parasite occupies only the horny epithelium of the nail bed, which has been thickened and split into layers by the action of the parasite. In addition, there is thickening of the stratum germinativum of the bed and hypertrophy of the papillary ridges. Only the terminal processes of the mycelium extend as far as the most superficial areas of the non-cornified stratum germinativum of the nail bed and the body of the nail is never invaded. In some cases the stratum germinativum of the nail bed may show some small concavities filled with mycelium, suggesting an incipient scutulum.

Fabry (18) and Fischer (19) concur with Unna that the parasite is mainly observed in the thickened horny epithelium of the nail bed and does not pass beyond the stratum germinativum, but Fischer states that he has found that the parasite may penetrate to the deeper layers of the body of the nail, giving rise to small opaque patches, observable even by macroscopic examination. He also draws attention to the presence of tubes which are empty owing to the death of the fungus and within which colonies of micrococci have grown.

Alkiewicz (14) in a histological study also found these tubes filled with air to be present in the body of the nail, producing the white network observed in the lesions described by this author.

White and Laipply (20) state that the histological changes of the nails in onychomycosis are similar to those observed in psoriasis, describing the thickening of the body of the nail and the presence of cystic nuclei and spaces in the subungual keratin as characteristic lesions.

Stühmer (13) found one of his cases of onychomycosis to be marked by parakeratosis of the most superficial layers of epithelium of the nail bed and by the presence of cavities filled with eleidin, situated between these layers and clumps of eleidin in the epithelium of the nail bed.

Sagher (21) has drawn attention to the differences in the distribution of *T. purpureum* and *T. violaceum* in the affected nails. The former invades the deeper layers of the nail or at most the intermediate layers and never the superficial layers, causing marked changes in the body of the nail and wide fissures. The latter, however, invades the more superficial layers of the body of the nail; no changes occur in the nail tissue surrounding the hyphae and the infection tends to disappear spontaneously.

5. Another matter to be explained is why the examination of nails showing clinical changes apparently due to fungus infection is frequently negative for fungi and why cultures cannot be made in cases in which direct microscopic examination has been positive for fungi. As regards the latter problem, Negroni (8) has stated that the parasite has come to be so closely adapted to the nail tissue, that it cannot grow on any other medium.

6. As for the pathogenic rôle of the fungi isolated in cases of onychomycosis, we refer to Benedek (22), who claims that the only part played by the dermatophytes isolated from affected nails is that of nosoparasites, as evaluation of their etiological role shows that Koch's postulates have not been complied with. Benedek's theory is supported by the fact that pathogenic fungi may even be found in normal skin and nails (Williams and Barthel (23), Jessner and Kleiner (24)). Benedek, however, having taken into account the positive results of inoculation obtained by Goetz (25), results obtained by auto-inoculation of cultures beneath the body of the nail, believes that there may have been some genuine cases of onychomycosis caused by dermatophytes.

According to Simons (26), the following mechanisms may be operative in the pathogenesis:

(a) Genuine primary onychomycosis, in which the fungus is the true causative organism. (b) "Onychomycotization", in which the affected nail has been secondarily invaded by pathogenic fungi. (c) "Pseudo-onychomycotization", in which there is secondary invasion of the nail by non-pathogenic fungi.

More doubtful, however, than the role of the dermatophytes, is the part played by fungi commonly regarded as saprophytes (*Scopulariopsis*, *Cephalosporium*, *Aspergillus*, *Hemispora*, *etc.*).

In regard to the above problems, we believe that experimental studies on human subjects might possibly elucidate some of the obscure points encountered

in the study of onychomycosis, especially when bearing in mind the fact that so far very little has been done in this field, as, to our knowledge and with the exception of Goetz's auto-inoculations, the only experiments made have been the attempts at inoculation undertaken by Wirz (cited by Miescher (2)), who, using material obtained from a nail lodging a large number of fungi, was unable to inoculate either the patient himself or others, not even when maceration with adhesive plaster or rubbing with sand-paper was done in preparation for the inoculation.

MATERIALS AND METHODS

1. *Subjects inoculated*, 14; 11 men and 3 women, from 13 to 72 years of age. None showed symptoms of diabetes. Of these patients, 2 were affected with tabes, 6 with epithelioma of the skin, 1 with allergic dermatitis, 1 with lupus tuberculosus, 1 with dermatitis herpetiformis, 1 with lichen due to arsenical poisoning, 1 with favus of the scalp and nails and 1 with pellagra.

2. *Nails inoculated.* Only normal finger-nails.

3. *Number of inoculations made*, 216. Various nails were inoculated by different methods and with different fungi in nearly all subjects.

4. *Fungi inoculated.* The following fungi were used: *T. schoenleini*, *T. rosaceum*, *T. violaceum*, *T. gypseum*, *T. tonsurans*, *T. rubrum*, *T. acuminatum*, *M. gypseum*, *Candida albicans*, *Cephalosporium.*

5. *Material inoculated.* We employed the scutula of favus, scales obtained from human subjects and lodging a large number of specimens of *T. rubrum*, filings from nails affected with onychomycosis due to *Cephalosporium* and *T. tonsurans* and primary cultures or cultures recently reinoculated into Sabouraud's medium of the 10 species employed.

6. *Inoculation technic.* The material of human origin and that obtained from cultures were mixed with honey and applied to the region inoculated for a period of 8–15 days by a bandage of cellophane and adhesive plaster.

The inoculation was made by simple deposit at the angle formed by the nail groove at the junction between the free margin of the nail and the tip of the finger in 50% of the cases and after superficial scouring with sand-paper in the others.

All inoculations into the free surface of the body of the nail were made after previous scouring with sand-paper.

We confined ourselves to depositing the mixture of fungi and honey in the two lateral nail grooves.

At the level of the lunula, in 75% of the cases the material to be inoculated was deposited in the angle formed by the lunula and the nail fold without any preliminary manoeuvre and after scouring of the lunula with sand-paper in the others.

Material was inoculated into the nail bed through the nail by an oblique cut made with the bistoury; after the entire body of the nail had been incised, the knife reached the nail bed, evidence of which was provided by the slight pain experienced by the patient at that moment. Hemorrhage was avoided in every

case. These incisions were made in various regions of the body of the nail, ranging from the lunula as far as the free margin. Goetz's method was used in one case, the virulent material being applied with a needle inserted parallel to the body of the nail, starting from the nail groove; this inoculation failed.

7. *Reading the results.* The subjects were examined for the first time by removal of the occlusive dressing covering the site of inoculation. Subsequently, they were examined every 15 to 30 days, from the clinical, subjective, objective and biological point of view, by direct microscopic examination and by cultures made in Sabouraud and Georg's medium. The examination could be completed within 180 days, or over in 151 inoculations. Examination was discontinued within 30 to 160 days in the others, owing to death from intercurrent disease in some cases and because the subjects were not seen again in others.

RESULTS

It is interesting to study the results from two points of view: 1) according to the fungus inoculated and 2) depending on the site where the inoculation was made.

1. Variation of the results with the fungus inoculated

A. *T. schoenleini.* The material inoculated was obtained from cultures in 48 cases and from powdered scutula of favus in 15. Of 22 inoculations made into the nail groove, only 4, in which powdered scutula of favus were inoculated, resulted in exfoliative superficial perionyxis, initially appearing at the tip of the finger, then spreading to the skin surrounding the nail and disappearing spontaneously within 20 to 50 days. Examinations for fungi were positive in all these cases of perionyxis. Nine inoculations with material from cultures and 6 with powdered scutula of favus into the body of the nail (upper surface) failed to induce any lesions. Application of the cultures to the lateral nail grooves in 7 cases also failed. However, exfoliative perionyxis and mycelium appeared in 2 cases, spread to the skin surrounding the nail and then disappeared spontaneously within 6 and 8 weeks respectively.

Six inoculations of material from cultures and 9 of powdered scutula of favus were made into the supra-ungual groove or angle. Both types of inoculation resulted in painful inflammatory superficial exfoliative perionyxis, appearing within 8 to 15 days in 11 cases. Some cases were complicated by vesico-pustular lesions, from which pure cultures of *T. schoenleini* were obtained. In these, there was desquamation in large sheets of epidermis, and the lesions disappeared spontaneously within 15 to 50 days (Figs. 1 and 2). The perionyxis recurred within 75 days in only one case (Fig. 3), after onychomycosis had appeared. The perionyxis again disappeared spontaneously within 4 weeks.

Of these 11 cases of perionyxis, 9 subsequently developed ungual lesions, and of the 4 showing no perionyxis, only one had marked onychomycosis. The ungual lesions were characterized by an identical course and development in all cases, varying only in severity. The first evidence of change was a punctiform or larger white spot, appearing on the lunula, exactly in the area covered by the epo-

FIG. 1

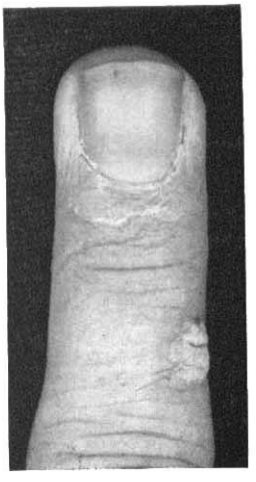


FIG. 2

FIG. 3

FIG. 1. Perionyxis marked by erythema and exfoliation within 10 days after inoculation of particles of the scutula of favus into the supra-ungual groove.

FIG. 2. Desquamative perionyxis in the stage of spontaneous disappearance within 25 days after inoculation of particles of the scutula of favus into the supra-ungual groove.

FIG. 3. Perionyxis recurring after 75 days and complicating the fully developed onychomycosis following inoculation of the scutula of favus into the supra-ungual groove.

nychium, within 8 to 15 days, but hardly ever exceeding a surface area of 4 sq. mm. This lesion soon became stationary and was followed by invasion of the nail throughout the entire lunula. The mycelium, which had penetrated deeply, invaded the root of the nail, altering its structure, appearance and consistency. The lunula was invaded by deep-seated white arborizations. Within 30 to 60 days, the most proximal portion of the nail showed some depressions, its consistency had become much softer, it was more deeply pink in color and it was interspersed with the white arborizations previously described. The depressions were cup-shaped, dotted or groove-like. When 2 to 4 months had elapsed, normal nail tissue started to grow and the affected portion, which had been pushed towards the free margin, on approaching the latter became detached from the bed, ulcerated and exfoliated in the greater part of the cases. The nail showed a completely normal appearance within 6 to 9 months. The results of examinations for fungi during the course of this experimentally-induced onychomycosis were extremely remarkable. Fungi were detectable by direct examination and cultures so long as the lesion had not spread as far as the middle of the nail, but from that time on biological examination became increasingly difficult as the lesion approached the free margin of the nail, until it had reached the inoculated area at the distal margin, examination of the nail being constantly negative for fungi in the stage of exfoliation (Figs. 4–10).

The severity of these ungual changes varied with the individual and even with the nails of a single subject. Thus the invasion of the nail in Fig. 10, showing the onychomycosis within 135 days after inoculation into two nails of the hand of a

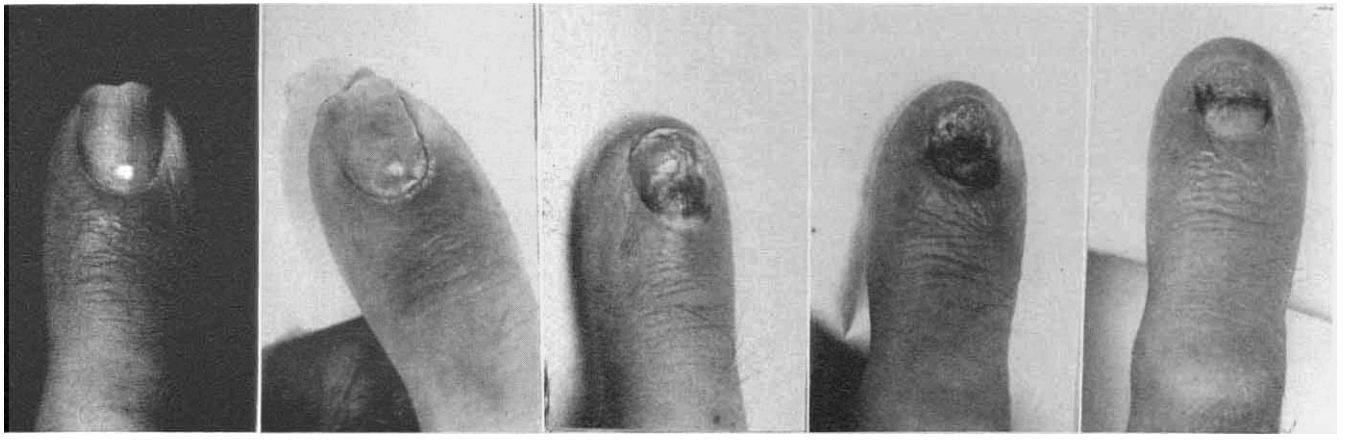


FIG. 4. Serial photographs showing the lesions induced by inoculation of the scutula of favus into the supra-ungual groove. (A) Superficial white patch, appearing within 40 days after inoculation of the scutula of favus into the supra-ungual groove and after the earlier perionyxis, shown in Fig. 1, had disappeared. (B) This white patch still persists after 50 days, but other white and arborescent lesions have appeared in the region of the lunula. (C) After 135 days, white patches have spread to the median area of the nail. Deep-seated chestnut- and purple-coloured spots have appeared in the posterior portion of the nail adjacent to the lunula. Normal nail is growing above the lunula. (D) After 180 days, almost the entire nail bed has become hyperkeratotic and shows friable white patches. (E) After 210 days, the posterior half of the nail is completely normal. The hyperkeratosis of the nail bed persists in the anterior half, although examination of this portion is negative for phytoparasites.

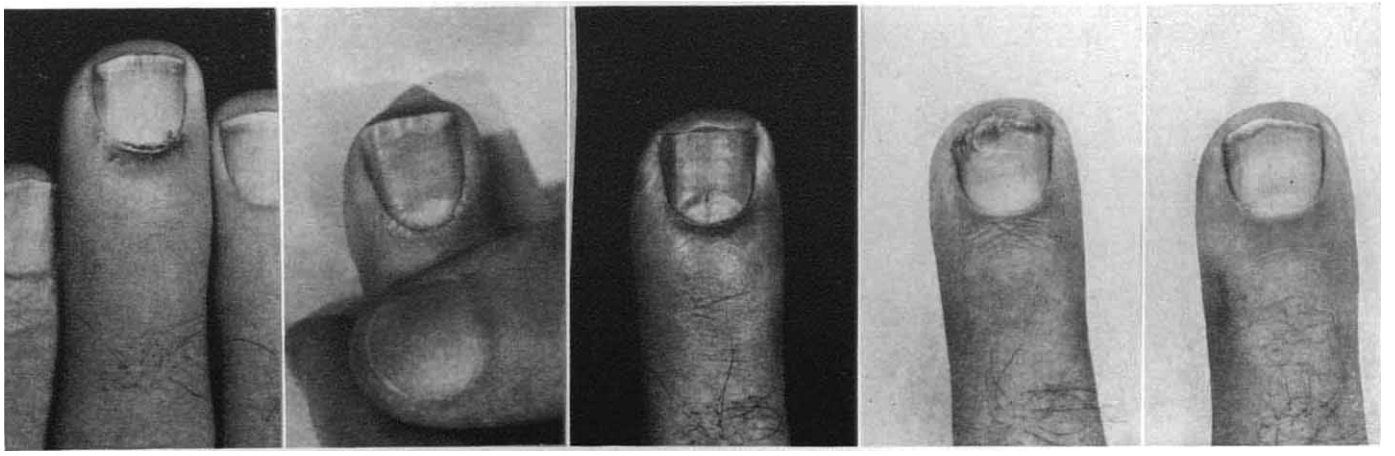
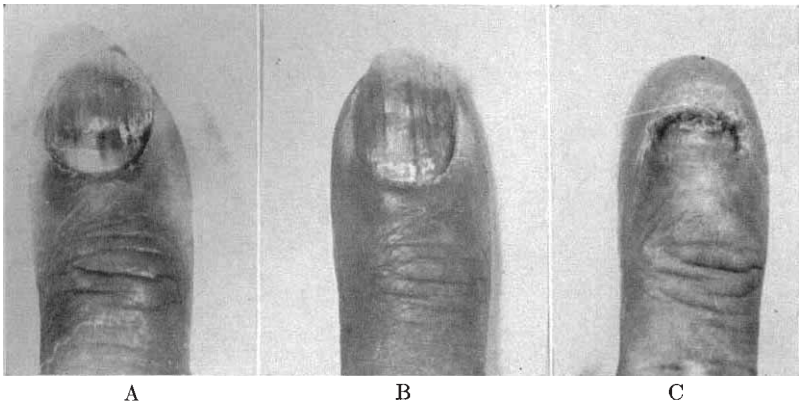
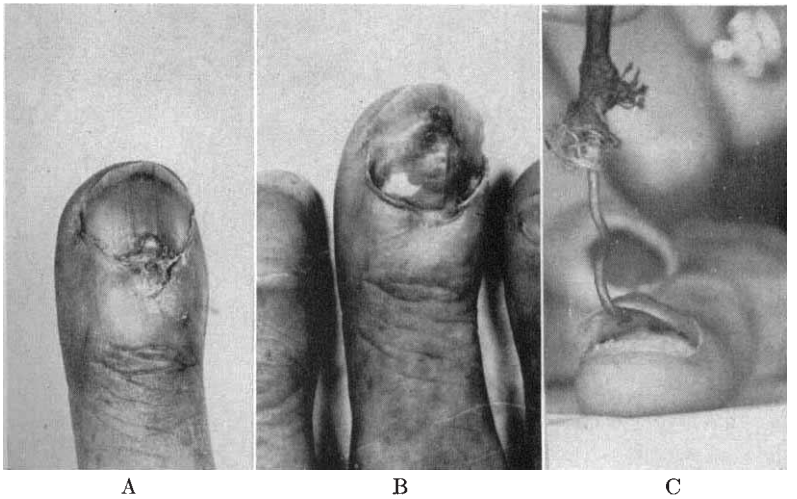


FIG. 5. Showing the course of lesions induced by inoculation of material obtained from cultures of *T. schoenleini* into the supra-ungual groove, after preliminary scouring of the region of the lunula with sand-paper. (A) Slight perionyxis within 20 days after inoculation. (B) Mild perionyxis persists within 45 days after inoculation. Slightly penetrating white spots and softening in the region of the lunula. Incipient longitudinal ulcer in the region of the lunula. (C) The perionyxis has disappeared within 57 days after inoculation; the nail is normal, although showing transverse grooves. (D) After 135 days, the ungual ulcer has started to disappear and has ascended as far as the free margin. Behind it, the transverse lines are being pushed forward by the normal growth of the nail body. (E) After 225 days, the nail is completely normal.



A B C

FIG. 6. Showing the course of the lesions following inoculation of the powdered scutula of favus into the supra-ungual angle. (A) The residual exfoliation of perionyxis and white spots due to superficial invasion by mycelium are still observed in the median portion of the nail within 75 days after inoculation. Deep-seated lesions of the matrix, dull white in colour, are seen through the root of the nail. These lesions are more marked in Fig. B. Examination is positive for fungi in either stage. The nail was excised within 135 days with a view to histopathological examination. Within 2 months after excision, a normal nail had grown, which had slipped over the hyperkeratotic bed, as shown in Fig. C.

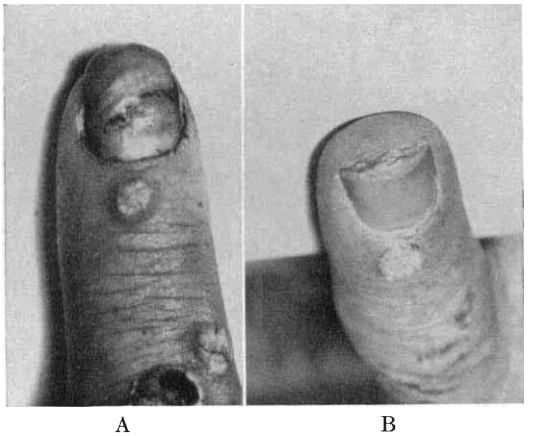


A B C

FIG. 7. Course of the lesions following inoculation of material obtained from cultures of *T. schoenleini* into the supra-ungual angle, biopsy of the eponychium being done within 6 days after inoculation. Histological examination of the specimen failed to reveal mycelium or spores. (A) The injury caused by biopsy had healed within 70 days after inoculation and the nail showed a dome-shaped enlargement at its root. (B) After 135 days, a marked snow-white spot has appeared on the posterior third of the nail, direct examination being positive for fungi, whereas cultures are constantly negative (dead mycelium ?) both in Sabouraud's and in Georg's medium. (C) Total onycholysis, exposure showing the nail bed to be hyper-keratotic.

A B

FIG. 8. Inoculation of the scutula of favus into the supra-ungual groove. (A) White spots situated in the thickness of the body and localized in the median portion of the nail are observed within 120 days after inoculation. Deep-seated punctiform lesions the color of dark chestnut have appeared in the region of the lunula. (B) After 210 days, the punctiform lesions penetrating into the region of the lunula have spread as far as the median portion of the nail and show a transverse arrangement. The white spots are disappearing in the region of the free margin.



A B

FIG. 9. Inoculation of material obtained from cultures of *T. schoenleini* in the region of the supra-ungual groove. (A) After 150 days, white patches have appeared in the thickness of the nail body, accompanied by a number of transversely directed ulcerative areas following elimination of a portion of the friable body of the nail in this region. (B) After 225 days, hyperkeratosis has appeared distal to the portion eliminated, as the lesions have spread as far as the free margin of the nail.

single subject, was deep-seated and resulted in destruction of the body of the nail of the left hand, whereas the lesions induced experimentally into the nail of the right hand did not advance beyond the first stage, i.e. that of the superficial white spot.

B. *T. rosaceum*. The material inoculated was obtained from cultures. The total number of inoculations was 25. Of these, 4 were made into the subungual

groove, 10 into the lateral grooves and 1 over the body of the nail; all these inoculations failed. Six were made into the supra-ungual angle, in the region of the lunula. Superficial exfoliative perionyxis, which disappeared spontaneously within 25 days, occurred within 15 days in only one case and it was only in this case that after 30 days small, superficial, dull white spots were seen to appear on the most proximal portion of the nail, direct examination and cultures being positive for mycelium, which had been eliminated by the usual contacts and washings 4 weeks later. Material was inoculated into the nail bed in 4 cases. Within 8 to 30 days a white fringe appeared at the bottom of the cut in these

FIG. 10. Two different types of ungual lesion in two nails belonging to the same hand. Scutula of favus were inoculated into the supra-ungual groove of the two nails on the same day and by an identical method. After 135 days, the two types of lesion are markedly different. The nail on the left side of the photograph shows almost complete destruction of the body of the nail, the nail bed being hyperkeratotic and showing desquamation. That on the right side shows only multiple white spots at various levels, which have not penetrated deeply into the thickness of the body.

cases, the fringe being closely confined to this region throughout the time it took to be eliminated spontaneously with the growth of the nail (Fig. 11). This white spot retained mycelium from which cultures could be made until its elimination.

C. *T. violaceum*. The material inoculated was obtained from cultures. The total number of inoculations was 20. Of these, 4 were made into the subungual groove, 4 into the lateral grooves and 12 into the body of the nail. All these inoculations failed.

Of 5 inoculations made at the supra-ungual angle, only one induced a giant Beau line, examination being positive for mycelium within 30 days. The patient died from multiple epithelioma 2 months later, when normal nail tissue appeared behind Beau's line. The two inoculations into the nail bed also failed to induce any changes.

FIG. 11

FIG. 12

FIG. 11. Inoculation of material from cultures of *T. rosaceum* through an oblique incision made in the median portion of the body of the nail and extending down to the nail bed. After 20 days, a white fringe, closely confined to the region of the incision and disappearing with the growth of the nail, is observed. Examination and cultures were positive for mycelium throughout the course of the lesions.

FIG. 12. Inoculation of material from cultures of *T. gypseum* at the supra-ungula angle. No perionyxis occurred, but after 15 days whitish dots due to invasion by mycelium are observed on the lunula, the lesions having fused after 50 days to form the large snow-white patch covering the lunula and extending beyond it, direct examination and cultures being positive for mycelium.

D. *T. gypseum*. The material inoculated was obtained from cultures. The total number of inoculations was 17. Of these, 3 made into the subungual groove failed to induce any lesions. Of 6 made into the lateral grooves, 3 also failed. During the second week the 3 others gave rise to a superficial proliferation of mycelium, invading only the most superficial layers of the nail and appearing as small white spots localized on the portion of the nail most proximal to the lateral grooves, which were eliminated by contacts and washings in 2 months at most.

Material was inoculated into the supra-ungual angle of two nails. After 15 days, almost the entire body of one of the nails was seen to be covered with small superficial yellowish white confluent patches, which increased and gradually extended to form a large uniform spot, marked by sharply defined and highly irregular borders (Fig. 12), within 50 days after inoculation. Within a few weeks, however, this striking lesion was gradually eliminated by contacts and washings. At the same time the nail was suddenly destroyed and, instead of growing normally, it was flattened, being composed of friable and brittle material, direct examination and cultures of which revealed the presence of mycelium. The other case ran an identical course, which was, however, preceded by inflammatory and desquamative perionyxis, appearing within 15 days after inoculation. Within 3 months, the two infected nails were destroyed as far as the free margins, normal nail tissue growing in the region of the matrix (Fig. 13 A: 34 days after inoculation; Fig. 13 B: 2½ months after inoculation).

Of 6 inoculations made into the nail bed, 5 were successful and 1 failed. Of

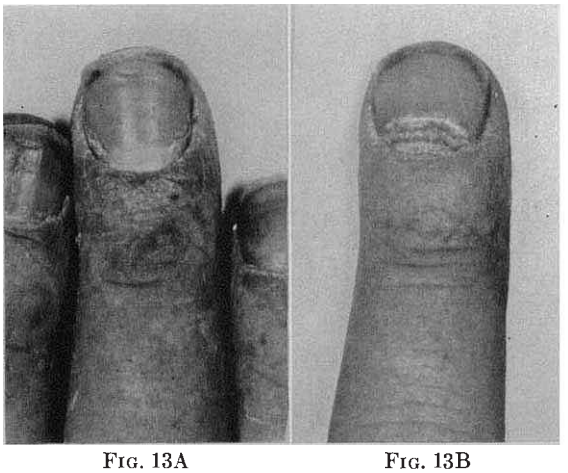


FIG. 13A FIG. 13B

FIG. 14

FIG. 13. Inoculation of material from cultures of *T. gypseum* into the supra-ungual groove, resulting in perionyxis within 15 days, which disappeared spontaneously after 45 days. (A) The sequelae of perionyxis are still observed within 34 days after inoculation, a transverse band of deep-seated parasitic invasion starting to appear in the most proximal portion of the lunula. (B) After 75 days, the nail shows transversely arranged white bands alternating with dark-coloured bands, of a softer consistence and composed of brittle material. These lesions are due to intermittent onsets of mycelian growth, the intervals enabling discontinuous growth of the least affected portion of the nail.

FIG. 14. Inoculation of material from cultures of *T. gypseum* through an oblique incision over the lunula, extending as far as the nail bed. Within 70 days, the nail shows a wide transverse line, flattened in front of the lunula. The body of the nail is very soft and exfoliates in the region of this line. The most anterior line, which passes transversely through the entire nail and which is very narrow, coincides with the line of inoculation. It shows that the growth of the fungus is obstructed by the side of the nail body and that it can develop only in the region of the lunula.

the successful inoculations, those made in the region of the lunula induced a rapid and extensive growth of mycelium, which appeared as extensive yellowish white spots accompanied by marked changes of the nail body, which became flattened, soft and friable (Fig. 14). The inoculations made near the free margin gave rise to an infection confined to the horny tissues altered by the incision. All these lesions were eliminated with the growth of the nail. Normal nail started to grow 3–5 months later. The only failure was the case in which the inoculation was made by Goetz's method.

E. *T. tonsurans*. The material inoculated was obtained from cultures. Inoculations were made in 15 cases. Three inoculations, all of which failed, were made into the subungual groove. Six inoculations were made into the lateral grooves. Of these, 2 gave rise to very small infected patches, solely confined to the softer horny tissue constituting the lateral margin of the nail, which disappeared spontaneously within 30 to 60 days. In the 2 other cases, the above lesions were preceded by an exfoliative perionyxis, which disappeared spontaneously.

An inoculation into the supra-ungual angle, and therefore in the region of the lunula, was made in 2 cases. One failed. Perionyxis, followed by deep invasion

FIG. 15

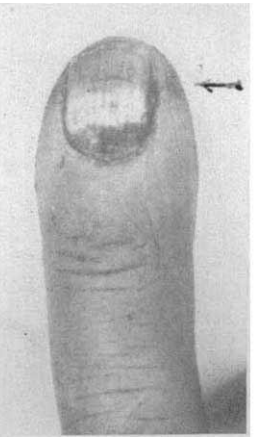


FIG. 16

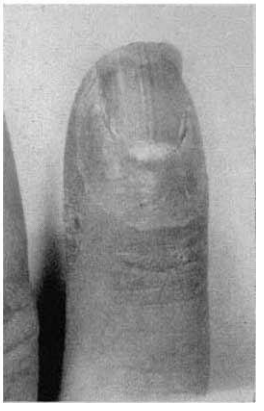


FIG. 17

FIG. 15. Inoculation of material from cultures of *T. tonsurans* into the supra-ungual groove. Within 75 days, an extensive white patch has appeared, penetrating the entire thickness of the nail. The appearance of the lunula behind this patch has changed, presenting a dark colour, being highly friable and desquamating.

FIG. 16. Inoculation of material from cultures of *T. tonsurans* through an oblique incision made in the region of the lunula and extending as far as the nail bed. After 90 days, the white patch due to invasion of the fungus is found to be localized behind the incision through which the inoculation was made. The nail between this patch and the root is very dark in colour, flattened, tapering and scaly.

FIG. 17. Inoculation of material from cultures of *T. rubrum* into the supra-ungual groove. After 30 days, there still is some evidence of perionyxis and a white patch resulting from the invasion of mycelium, which covers and has penetrated the thickness of the entire lunula.

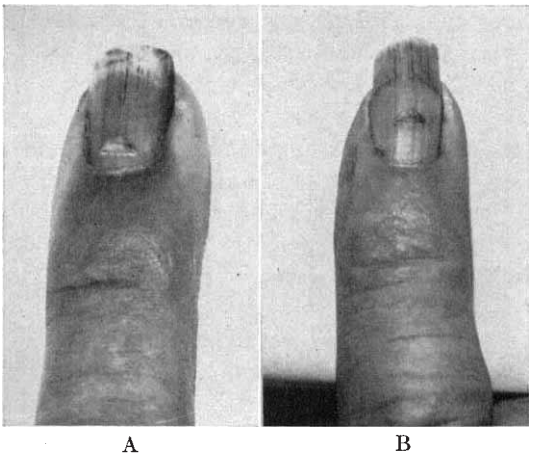
of the nail, appeared within 8 days in the other case. The lunula assumed a dull color, lost its gloss and became soft, showing a moth-eaten appearance (Fig. 15, after 2½ months). Direct examination and cultures were positive for mycelium. Subsequently, the most proximal portion of the lunula was flattened, with softening of its horny tissue, which lodged a large number of parasites. This patient was lost sight of after 3 months, but when the effects of inoculation could be examined again 9 months later, the nail was found to be completely normal.

Of 4 inoculations made into the nail bed by incision, 2 were successful and 2 failed. The most marked lesions were induced by inoculation by incision into the lunula (Fig. 16). The other lesions, appearing in the median portion of the body of the nail, were very slight, softening occurring only at the site of the incision into which the inoculation was made, with a very slight spread in the region of the nail bed. The inoculations made near the free margin of the nail failed to induce any lesions.

F. *T. rubrum*. The material inoculated was obtained from cultures or from scales of a dermatophytosis caused by *T. rubrum*. The total number of inoculations was 34. Eight inoculations were made into the subungual groove, material from cultures being inoculated in 4 cases and material from scales in the other 4.

Material from cultures was inoculated into the lateral grooves in 4 cases. Four inoculations were made by applying the material to the upper surface of the nail body, material from cultures being used in 2 cases and material from scales in the other 2. So far, all inoculations have failed. Ten inoculations were made into the supra-ungual groove, material from cultures being employed in 6 cases and material from scales in 4; only one of the inoculations of material from cultures gave rise to lesions, initially appearing as a transient exfoliative superficial perionyxis, followed 15 days later by invasion of the lunular area of the body of the nail, in the form of a white patch involving the entire thickness of the nail, examination of the lesions being positive for fungi (Fig. 17, after 30 days). The perionyxis disappeared within 3 weeks, whereas the white patch on the lunula spread, becoming soft and flattened as it increased; after 30 days the patient was no longer examined, as he had left the hospital. When seen again 9 months later, the nail was normal.

In making the 8 inoculations into the nail bed, only material obtained from cultures was employed. The inoculations made near the free margin of the nail failed to induce any lesions. The lesions appearing in 4 cases consisted in an extensive white patch in the region of the nail bed, which could be seen through the body of the nail; its growth rate and size increased with the proximity of the site of inoculation to the lunula, its growth and size being most marked when the inoculation was made into the matrix proper (Fig. 18 A). Despite the spread of mycelium towards the lunula, dissemination of the parasites ceased within 2½ months (Fig. 18 B); finally, the lesions were eliminated within 5–6 months with the growth of the nail.



A B

FIG. 18. Inoculation of material from cultures of *T. rubrum* by a transverse incision into the body of the nail, approaching, but not reaching the lunula. (A) After 15 days. (B) After 75 days. The two photographs clearly show the invasion of the nail bed by the dermatophyte, seen as a transverse white line situated at the proximal edge of the incision, i.e. at its base. Despite the fact that cultures are positive for fungi, proliferation of the mycelium is confined to the incision without spreading to the rest of the nail until it is expelled on reaching the free margin.

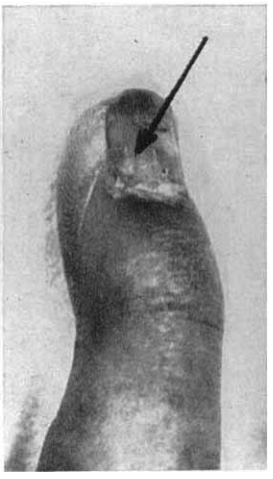


FIG. 19

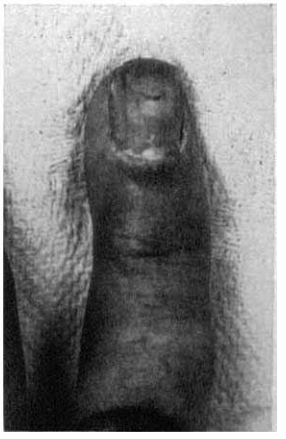


FIG. 20

FIG. 19. Inoculation of material from cultures of *M. gypseum* by application to the lateral nail groove. After 40 days, a superficial small whitish patch is observed; direct examination and cultures are positive for mycelium, the patch disappearing spontaneously within 45 days as a result of the usual contacts and washings.

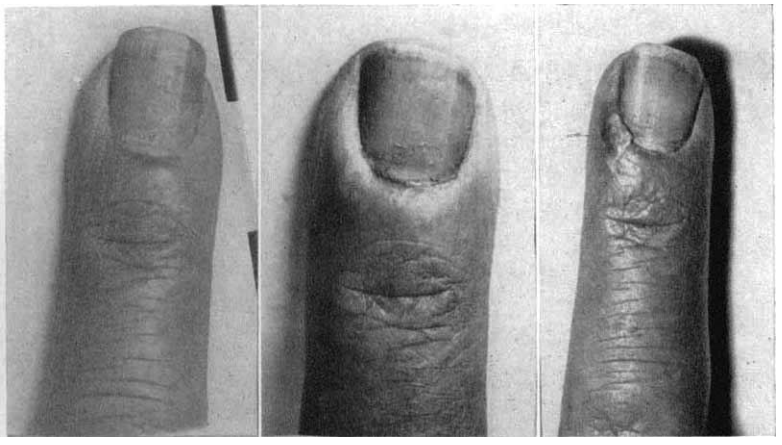
FIG. 20. This nail shows the result of inoculation of material from cultures of *M. gypseum* through a transverse incision into the body of the nail and that of simultaneous inoculation into the supra-ungual groove. Within 50 days, being concerned only with the inoculation made into the supra-ungual groove, snow-white, sharply defined patches, invading and extending slightly beyond the lunula, are observed.

G. *M. gypseum.* Material obtained from cultures was inoculated into 22 nails. Four made into the subungual groove and 2 in which the material was applied to the upper surface of the body of the nail failed. Of 9 inoculations made into the lateral grooves, 7 gave rise to lesions, the appearance and course of which were similar to those of the lesions previously induced by *M. gypseum.* Examination of the lesions was positive for fungi (Fig. 19).

The 3 inoculations made at the supra-ungual angle all were clinically and biologically positive, giving rise to changes, the appearance and course of which were similar to those induced by *M. gypseum* (Fig. 20, within 50 days after inoculation; Fig. 21 A, after 15 days; Fig. 21 B, after 20 days and Fig. 21 C, after 30 days).

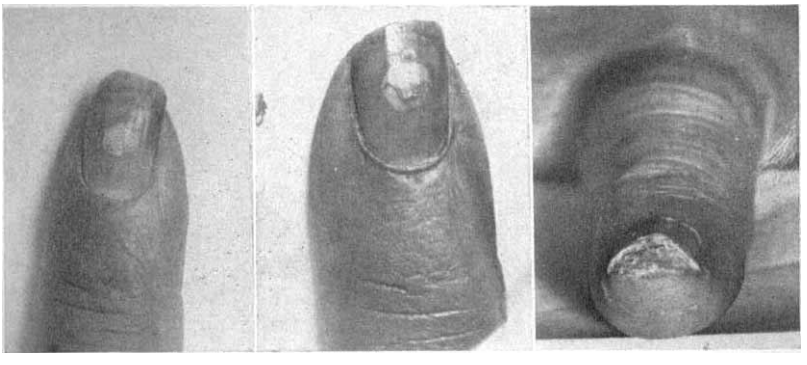
Four inoculations were made into the nail bed, all of which gave rise to lesions within 8–15 days. The white patches were more extensive and more marked than those induced by the other fungi inoculated and they also were more marked, the closer to the lunula the inoculation was made (Figs. 22 A, 22 B and 22 C, within 15, 20 and 50 days after inoculation respectively). As was seen to occur in the case of other fungi, the lesions were eliminated spontaneously by the growth of the nail.

H. *Candida albicans.* The material inoculated was obtained from cultures. The total number of inoculations was 10. The 2 inoculations made into the subungual groove gave rise to a very mild perionyxis marked by erythema and



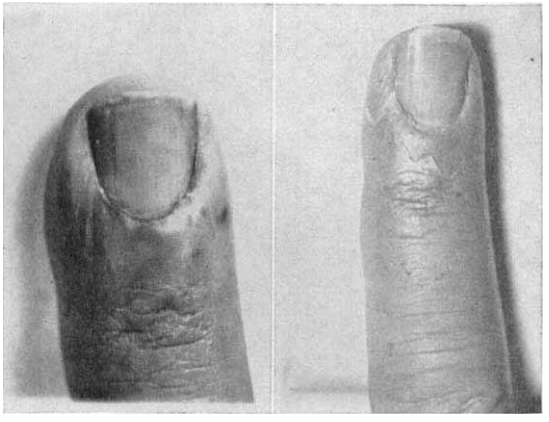
A B C

FIG. 21. Inoculation of material from cultures of *M. gypseum* into the supra-ungual groove. (A) After 15 days, there is a very superficial invasion of the lunula and the proximal portion of the nail body, which show slight evidence of desquamation, examinations being positive for fungi. (B) After 20 days, the most superficial portion of the nail has been eliminated spontaneously, leaving a very shallow depression as a result. Perionyxis has appeared. (C) After 30 days, the perionyxis has disappeared, the only evidence of disease shown by the nail body being the flattened, abnormally soft and dull lunula.



A B C

FIG. 22. Inoculation of material from cultures of *M. gypseum* through an oblique incision into the median area of the body of the nail. (A) Within 15 days after inoculation, the invasion of the nail bed by mycelium has given rise to a round white patch, seen clearly through the nail body. (B) After 20 days, this patch has become stationary and, along with the incision through which the inoculation was made, it has been driven towards the free margin by the growth of the nail. (C) After 50 days, the onycholytic area of the free margin is cut again with the scissors, to expose more clearly the pulverulent subungual hyperkeratosis. Direct examination reveals abundant mycelium, cultures of which provide pure colonies of *M. gypseum*. In addition, there is an exfoliative collarette of the finger-tip, infected with the mycelium of the body of the nail during its elimination. This disappeared spontaneously within 30 days. From that time, examinations both of the nail and of the tip of the finger were constantly negative for fungi.



A B

FIG. 23. Inoculation of material from cultures of yeasts (*Candida*) into the supra-ungual groove. (A) After 20 days, perionyxis marked by erythema and vesicles has appeared, the surface of the skin being brilliant and tense. Pure colonies of *Candida* are obtained from the fluid of the vesicles. (B) After 30 days, exfoliation of the affected surface of the skin in large plates is observed. No changes have occurred in the nails, with the exception of a slight and transient superficial maceration of the lunular area.

vesico-pustular lesions, observed when the protective bandage was removed 8-days later, the lesions disappearing spontaneously within 30 to 50 days (Fig. 23 A, after 20 days). There was no invasion of the nail. Of 3 inoculations made into the lateral grooves, only 1 gave rise to a transient perionyxis similar to that induced in previous cases (Fig. 23 B, after 30 days). Two inoculations made into the upper surface of the body of the nail, 2 made at the supra-ungual angle and 1 made into the nail bed failed to induce any lesions.

I. *T. acuminatum*. Only one inoculation was made by incision into the median portion of the nail bed, which failed to produce any lesions.

J. *Cephalosporium*. The material inoculated was obtained from cultures and filings of the nails in a case of onychomycosis spontaneously induced by this fungus. Two inoculations of material obtained from cultures and two of filings of an infected nail into the subungual groove failed to induce any lesions, as was also the case with 2 inoculations made at the supra-ungual angle.

DISCUSSION AND SUMMARY

The uncommon character of onychomycosis is reflected in the fact that experimental inoculations were successful only in a few cases. We were only able to induce lesions in 52 out of 216 cases, i.e. in 24 per cent of the cases.

Obviously, the susceptibility of the different areas of the nail varies markedly. The proportions in which lesions were induced by inoculation in each of these areas were as follows: no lesions in the body of the nail and subungual groove, in the lateral grooves in 28.5 per cent of the cases, in the supra-ungual groove in 40.4 per cent of the cases and in the nail bed in 63.3 per cent of the cases.

The incidence of the lesions induced by inoculations into the last-named area

and the severity of the clinical symptoms increased with the proximity to the lunula of the site of inoculation.

Obviously, the lunula was the most susceptible area, both when the inoculation was made by incision through the body of the nail and when the fungus penetrated in the region of the root, spreading around the latter.

Clinically, the lesions induced appeared as whitish spots in the greater part of the cases, thus bearing a resemblance to the forms of leukonychia due to fungi described in the introduction, which should be regarded as the initial form of onychomycosis. Other cases were characterized by a subsequent development of these lesions, marked by progressive destruction of the body of the nail as the infected area initially appearing in the lunula approached the free margin, the form of disease usually described in clinical studies.

In contrast with the prolonged duration of spontaneous onychomycosis, the disease never persisted beyond 180 days in our cases. This may have been due in part to the massive character of the experimentally induced infection, as the latter is likely to give rise to allergic and immunological reactions different from those resulting in the case of repeated and slight spontaneous inoculations.

During our inoculations we also observed marked variations in the severity of the lesions in a single individual and even in the nails of one hand into which the same fungus had been inoculated.

Direct examination and cultures continued to be positive for fungi in the experimentally inoculated nails, so long as the lesions appeared as whitish spots near the lunula; on the other hand, cultures started to become negative as the changes approached the free margin and the nail underwent a process of decay, the cultures finally being constantly negative for fungi. This explains in part why direct examinations are frequently positive in spontaneously infected nails, whereas cultures are negative; in part, as in a small number of cases the negative cultures may be due to the fact that they are made in media which do not exclude the simultaneous development of contaminants, a large number of which are present in the nails. We solved this problem by using a medium containing actidione, penicillin and streptomycin, as suggested by Georg (27), in the examination of spontaneous and experimentally induced infections of the nails.

In our opinion, there is no doubt as to the part played by dermatophytes in the pathogenesis, as the clinical pictures induced by inoculation showed constant morphological features similar to some of those described as occurring in clinical cases; and also as the presence of these organisms could be verified in the affected nail and as they could be isolated by cultures.

Our experiments have also shown that previous disease of the nail is not essential to the development of dermatophytes in the latter.

As regards contaminants, we inoculated one of them, a Cephalosporium, but failed to induce any lesions. In view of the small number of experiments, no conclusion can be deduced from our studies.

Our investigations have shown that when the nail is excised in treatment, it should be removed in its entirety, i.e. including the root, the site of onset and persistence of the infection.

It may be stated that onychomycosis and tinea capitis have several features in common. In either case infection is confined to certain areas in the vicinity of the root (see Kligman's experimental studies on microsporia (29)), which is possibly due to the fact that conditions of adequate humidity or chemical composition prevail in these areas. Like the hair, the nail decreases in consistence and disintegrates as the region involved moves away from the root during growth, the hair breaking off at a greater or smaller distance from the follicle and the nail prior to reaching the free margin. Finally, avulsion of the root is indicated in either case, the root of the hair being removed by epilation and that of the nail by surgical avulsion, without which no constant and permanent cure can be obtained.

It also is a mistake to believe that the increased severity of the lesions in the region of the free margin of spontaneously affected nails is evidence showing that the infection initially occurred at this site. This would be equivalent to thinking that e.g. the rupture and disintegration of the extrafollicular portion of the hair in microsporia is evidence of the fact that the infection initially appeared in this region and not in the root, as is actually the case.

2. Regarding the site where the inoculation was made

A. Inoculations into the upper surface of the body of the nail after preliminary scouring of the body with sand-paper.

Material obtained from cultures or from virulent human lesions was inoculated in 27 cases, divided as follows: material from cultures of *T. schoenleini* in 9 cases, scutula of human favus in 6, material from cultures of *T. rosaceum* in 2, material from cultures of *T. violaceum* in 2, material from cultures of *T. rubrum* in 2, material from scales of individuals affected with marked dermatophytosis due to *T. rubrum* in 2 cases, material from cultures of *M. gypseum* in 2 and material from cultures of *Candida albicans* in 2 cases.

No lesions or changes of the body of the nail were observed during an average period of 6 months.

B. Inoculations merely by application of material obtained from virulent human lesions or cultures of phytoparasites to the subungual groove.

Material containing *T. schoenleini* was inoculated in 22 cases, *T. rosaceum* in 4, *T. violaceum* in 4, *T. gypseum* in 3, *T. tonsurans* in 3, *T. rubrum* in 8, *M. gypseum* in 4, *Candida albicans* in 2, *Cephalosporium* (cultures) in 2 and filings infected with onychomycosis due to *Cephalosporium* in 2 cases.

All these inoculations, which were observed for 180 days, failed to produce any lesions. Only 4 nails into which scutula of favus had been inoculated showed a red superficial exfoliative perionyxis, which disappeared spontaneously within 20–50 days, without at any time invading the body or the nail bed. *Candida albicans* gave rise to very mild perionyxis with erythema and vesico-pustular lesions, observed when the bandage was removed after 8 days and disappearing spontaneously within 30–50 days. Nor were the nails and the nail bed affected.

C. Inoculations of material obtained from cultures into the nail bed through an oblique incision made with the bistoury in various regions of the body of the nail.

TABLE I

Fungi Inoculated	Number of Inoculations	Results Obtained Within							
		8 days	15 days	30 days	60 days	90 days	120 days	150 days	180 days
T. schoenleini	4	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
T. rosaceum	4	++	++	++	−−	−−	−−	−−	−−
		++	++	++	++	−−	−−	−−	−−
T. violaceum	2	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
T. gypseum	6	++	++	++	++	+−	−−	−−	−−
		++	++	++	++	−+	−+	−+	−−
		++	++	++	+−	−−	−−	−−	−−
T. tonsurans	4	++	++	+−	+−	+−	+−	+−	+−
		−−	−−	−−	−−	−−	−−	−−	−−
T. rubrum	8	−+	−+	−+	−+	−−	−−	−−	−−
		+−	++	++	++	++	−−	−−	−−
		−−	−−	−−	−−	−−	−−	−−	−−
		−−	+−	+−	+−	+−	−−	−−	−−
M. gypseum	4	−+	++	++	+−	−−	−−	−−	−−
		++	++	++	−+	−+	−+	−+	−+
C. albicans	1	−	−	−	−	−	−	−	−
T. acuminatum		−	−	−	−	−	−	−	−

T. schoenleini—Only the incision assumes a yellowish color. The incisions are eliminated spontaneously by the growth of the nail.

T. rosaceum—Slight spread to the nail bed. Narrow whitish band closely surrounding the incision through which the inoculation was made.

T. gypseum—The inoculations into the lunula give rise to extensive, rapidly growing spots which, having invaded the entire lunula, become stationary and are eliminated spontaneously by the growth of the nail. The inoculations through the rest of the body induce identical, but less rapidly growing and less extensive lesions. The only case in which lesions failed to appear was that in which the inoculation was made with a needle inserted from the free margin following anesthesia, Goetz's method being employed.

T. tonsurans—The inoculations made into the lunula gave rise to lesions similar to those induced by *T. gypseum*. They failed to produce any lesions in the rest of the body of the nail.

T. rubrum—The inoculations into the lunula rapidly resulted in total invasion of the latter, whereas those made through the body only caused lesions extending less than 0.5 mm. around the incision.

M. gypseum—Inoculations of this fungus rapidly induced extensive areas of infection, increasing with the proximity of the inoculation to the lunula. The lesions were also eliminated spontaneously by the growth of the nail.

All the cases showing lesions included in this table are cases in which the fungi penetrated to the deepest layers of the nail resting upon the nail bed. The changes were observed at the junction of the bed and the body of the nail. The body of the nail only showed gross lesions when the region of the lunula was invaded.

In all these inoculations, the variations in the time required by the lesions to

TABLE II

Fungi Inoculated	Number of Inoculations	Results Obtained Within							
		8 days	15 days	30 days	60 days	90 days	120 days	150 days	180 days
T. schoenleini	7	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
T. rosaceum	10	−−	−−	−−	−−	−−	−−	−−	−−
		−−	−−	−−	−−	−−	−−	−−	−−
		−−	−−	−−	−−	−−	−−	−−	−−
		−−	−−	−−	−−	−−	−−	−−	−−
		−−	−−	−−	−−	−−	−−	−−	−−
T. violaceum	4	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
T. gypseum	6	+−	+−	+−	+−	−−	−−	−−	−−
		+−	+−	+−	+−	−−	−−	−−	−−
		+−	+−	+−	+−	−−	−−	−−	−−
T. tonsurans	6	+−	++	++	++	−−	−−	−−	−−
		+−	+−	+−	−−	−−	−−	−−	−−
		+−	+−	−−	−−	−−	−−	−−	−−
T. rubrum	4	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
M. gypseum	9	+−	++	−+	−−	−−	−−	−−	−−
		−−	−+	−+	−+	−−	−−	−−	−−
		−−	−+	−+	−+	−−	−−	−−	−−
		+−	++	++	+−	−−	−−	−−	−−
		−	+	+	+	+	−	−	−
Candida alb.	3	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−

T. schoenleini—Superficial exfoliative perionyxis was induced in two cases.

T. gypseum—The lesions induced consisted in small white patches on the surface of the body of the nail, disappearing spontaneously within 1–2 months.

T. tonsurans—In addition to the small patches previously described, superficial exfoliative perionyxis was observed in two cases.

T. rubrum—No changes of the nails; a transient superficial perionyxis appeared in all cases, however.

M. gypseum—Direct examinations and cultures were positive for fungi in all cases showing lesions (small patches on the surface of the nail body).

Candida albicans—Superficial perionyxis, with vesicles and exfoliation, occurred in all cases and disappeared spontaneously.

disappear solely depended on the level at which they were made over the antero-posterior axis of the nail. The line of inoculation and the resulting lesions were eliminated with the growth of the body of the nail in each case.

When the inoculation gave rise to lesions and was made into the lunula or in its vicinity, resulting in subsequent invasion of the latter, the nail showed changes in composition and structure. During 30–60 days this altered nail was marked by a very broad transverse line, behind which normal nail started to grow again. In some cases, in which the inoculation was made close to the distal margin and failed to give rise to clinical symptoms, direct examination and cultures were positive for fungi for 45 days after inoculation; this was the case with *T. schoenleini*, *M. gypseum*, *T. gypseum* and *T. rosaceum*.

D. Inoculations of material obtained from cultures made merely by application to the lateral grooves. Total number of inoculations, 48.

TABLE III

Fungi Inoculated	Number of Inoculations		Results Obtained Within															
			8 days		15 days		30 days		60 days		90 days		120 days		150 days		180 days	
	1	c	1	c	1	c	1	c	1	c	1	c	1	c	1	c	1	c
T. schoenleini	9	6	++	++	++	++	++	++	++	++	++	++	++	++	++	+−	−−	−−
			++	−−	++	++	++	++	++	++	++	++	++	+−	−−	−−	−−	−−
			++	−−	++	−−	++	−−	++	−−	++	−−	++	−−	−−	−−	−−	−−
			−−		−−		−−		−−		−−		−−		−−		−	
			−		−		−		−		−		−		−			
T. rosaceum	—	6		+−		+−		+−		−−		−−		−−		−−		−−
				−−		−−		−−		−−		−−		−−		−−		−−
				−−		−−		−−		−−		−−		−−		−−		−−
T. violaceum	—	5		+−		+−		+−		−−		−−		−−		−−		−−
				−−		−−		−−		−−		−−		−−		−−		−−
				−		−		−		−		−		−		−		−
T. gypseum	—	2		−−		++		++		++		++		−−		−−		−−
T. tonsurans	—	2		+−		+−		+−		+−		+−						
T. rubrum	4	6	−−	+−	−−	+−	−−	+−	−−	+−	−−	−−	−−	−	−−	−	−−	−−
			−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−
				−−		−−		−−		−−		−−		−−		−−		−−
M. gypseum	—	3		++		++		++		++		++		++		++		++
				−		+		+		+		+		+		+		+
Candida alb.	—	2		−−		−−		−		−		−		−		−		−
										−		−		−		−		−
Cephalosporium	—	2		−		−		−		−		−		−		−		−
				−		−		−		−		−		−		−		−

T. schoenleini—Supralunar yellowish patches, with dullness and rugosity of the body of the nail in the region of these patches and on the surface. As these patches were eliminated by the growth of the nail, the matrix was invaded and a Beau line appeared, which was eliminated in turn, normal nail growing behind this line. The initial lesion was a painful exfoliative perionyxis, which healed spontaneously, in the majority of cases.

T. rosaceum—The only subject developing lesions died from dermatitis herpetiformis within 34 days.

T. violaceum—The only lesion was an enormous Beau line, normal nail growing behind this line.

T. gypseum—Small yellow-white patches, with flattening and softening of the lunula. Superficial exfoliative perionyxis appeared in one case.

T. tonsurans—One case showed lesions identical to those induced by *T. gypseum*, but examination was discontinued after 3 months. After 9 months, the nail was normal.

T. rubrum—Examination was discontinued after 30 days in the case showing lesions and examination was negative after 120 days in 3 cases.

M. gypseum—Small white patches on the surface of the lunula, a softened depression appearing behind these patches.

Candida albicans—Superficial perionyxis, with vesicles and exfoliation, disappearing spontaneously within 15–30 days.

E. Inoculations of material obtained from virulent human lesions (1) or cultures (2) by application to the supra-ungual groove (supralunular region). Total number of inoculations, 47.

Inoculations into the supra-ungual groove usually were followed by the rapid appearance of perionyxis with erythema and exfoliation, which healed spontaneously within 20 to 40 days. The primary lesions of the nail were vegetative in almost all cases. They were very superficial, only causing dullness of the surface of the nail body. At a later stage, the mycelium invaded the root of the nail, altering its structure and giving rise to the appearance of a Beau line. The nail subsequently became normal in all cases and the lesions were eliminated by the growth of normal nail.

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